PHOMA BETAE ON THE LEAVES OF THE SUGAR BEET

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INTRODUCTION

Various names have been given at different times to the fungus causing a root-rot, a damping-off, and a leaf-spot of the sugar beet (*Beta vulgaris* L.), and consequently their relationship to each other has not been recognized. The leaf-spotting was attributed by Oudemans[1] to *Phyllosticta betae* and later by Prillieux and Delacroix[2] to *P. tabifica*. Frank[3] believed the latter organism to be identical with his root-rot fungus, *Phoma betae*, but on account of generic differences no combination of names was made. Hedgcock[4] pointed out for the first time a definite connection between Phyllosticta on the leaf and Phoma on the root. Peters[5] and Edson[6] give evidence that the fungus which produces leaf-spotting is also a cause of damping-off. The present investigation shows that the leaf-spot and the root-rot organism are the same and points out that the entire life cycle of the fungus must be considered in any interpretation that is made of the disease phenomena. The name "*Phoma betae* (Oud.) Fr." is deemed by the writers and by Edson[6] to be correct and inclusive; however, the generic name "Phyllosticta" is retained in this paper for the organism isolated from leaves.

SYMPTOMATOLOGY

A mature, normally developed spot of *Phoma betae* on the sugar-beet leaf varies in size from 1 to 2 cm. in diameter and is usually light brown in color. At times such spots show concentric rings of growth, the different zones being outlined by pycnidia. There is no sharp differentiation (Pl. XXVII) between the infected area and the surrounding tissue, owing to the lessened activity of the beet leaf at the time the organism is growing in the leaf tissue. This accounts for the comparatively large size of the spot and its rather diffusive character. The spots which

[1] Oudemans, C. A. J. A. Aanwinsten voor de flora mycologica van Nederland van Juli 1875 tot Juli. 1876. *In* Nederland. Kruidk. Arch., s. 2, deel 2, stuk 3, p. 181. 1877.

[2] Prillieux, E. E., and Delacroix, Georges. Complément à l'étude de la maladie du cœur de la betterave. *In* Bul. Soc. Mycol. France, t. 7, p. 23–25, pl. 3. 1891.

[3] Frank, A. B. Phoma Betae, ein neuer Rübenpilz. *In* Ztschr. Pflanzenkrank., Bd. 3, p. 90–92. 1893.

[4] Hedgcock, G. G. Proof of the identity of Phoma and Phyllosticta on the sugar beet. *In* Jour. Mycol., v. 10, p. 2–3. 1904.

[5] Peters, Leo. Ueber die Erreger des Wurzelbrandes. *In* Arb. K. Biol. Anst. Land- u. Forstw., Bd. 8, Heft 2, p. 229–239. 1911.

[6] Edson, H. A. Seedling diseases of sugar beets and their relation to root-rot and crown-rot. *In* Jour Agr. Research, v. 4, no. 2, p. 135–168. 1915.

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first appear are generally brown, rarely red, in color. The latter color suggests that the lesion was first produced by some injury which probably caused the formation of carotin, the fungus later gaining an entrance. Typical spots grow rapidly and after 10 days or 2 weeks the black, somewhat erumpent pycnidia develop.

During the growing season of 1912 at Rocky Ford, Colo., observations were made of the different types of Phoma spots that were found to occur. Small brown spots were first noted on old mature leaves in the early part of July. Cultures made from such spots developed colonies of *Cercospora beticola* and *Phoma betae*. Several small red spots collected somewhat later gave either pure cultures of *P. betae* or a mixture of Phoma and an Alternaria. It would seem that the earliest spots contained more than one organism, owing probably to the fact that insect wounds made it easy for various fungi to enter. These spots frequently did not enlarge, showing that the organism had gained no sure foothold. By the last of July or the first part of August large, light-brown, typical spots yielded pure cultures of *P. betae*. Such spots always occurred on those leaves which were old and showed symptoms of yellowing. Consequently on a normal beet plant only a few leaves were infected, but on a plant that was physiologically weakened as a result of rot caused by *Rhizoctonia solani* or of some other factor inimical to plant growth many leaves were found to have typical spots. This observation was confirmed during the season of 1914 at Madison, Wis., where many of the leaves on the "mother beet" plants were found to be infected with Phoma. The roots from which these plants had grown had been more or less affected by various storage rots during the preceding winter, and consequently the vitality of the plants was greatly lowered.

The leaves attacked on the normal and abnormal plants showed the same symptoms of age. Thus, it would seem from field observations that the age of the leaf becomes the important factor in its susceptibility to the disease, and this is upheld in controlled experiments.

AGE AS A FACTOR IN LEAF SUSCEPTIBILITY

Practically all inoculation experiments carried on in 1912 to determine the connection between *Phoma betae* and *Phyllosticta betae* gave negative results. In all the preliminary studies the pycnospores of Phoma from the root and of Phyllosticta from the leaf were suspended in sterile water and either sprayed or smeared on leaves of all ages. Out of 150 inoculations thus made there were only four infections, and these occurred on old, yellow leaves, indicating that the organism is only rarely able to penetrate the unbroken epidermis. Later work has shown that even at the most favorable age the great majority of infections take place through some lesion on the leaf surface.

It was found, after making a large number of counts, that the maturity and the relative age of the different leaves of a beet plant could be determined by taking an average of the number of stomata on a given area at the base, middle, and apex of the leaf.[1] Numerous preliminary determinations showed that within certain ranges the number of stomata that occurred on either surface of the leaf was indicative of its age, so, for convenience, all subsequent counts were made on the upper surface. It was ascertained that leaves with 53 to 100 stomata per square milimeter could be considered as mature and were so designated. Every leaf in the outermost whorls on all plants examined gave stomatal counts within this range. Presumably the cells of such leaves had reached their maximum growth and their greatest metabolic activity. Young mature leaves which had a stomatal count per square milimeter of 92 to 133 were usually taken from a medium position on the plant and were metabolically active, although they had not as yet reached their greatest size. Leaves which had 134 or more stomata per square milimeter were very immature and were located near the heart growth of the plant.

In order to determine which were the most susceptible to infection by *Phoma betae*, 21 needle lesion inoculations on young leaves, 39 on medium-aged leaves, and 90 on old, mature leaves were made. Only 34 infections developed, and these were on the old, mature leaves. A comparable series of inoculations made with *Phyllosticta betae* gave 25 infections from 91 inoculations on old, mature leaves, no infections from 12 inoculations made on medium-aged leaves, and none from 42 made on young leaves. On the petioles 50 inoculations gave no infection with either organism. The number of infections was not increased when the plants were covered with bell jars or pots, but the infected areas appeared somewhat sooner than on uncovered plants. Typical spots developed, if at all, from two to four days after inoculating; however, this incubation time is in all probability lengthened under less favorable field conditions. (See Table I.)

[1] The stomatoscope originated by Prof. F. E. Lloyd was made available for the work through the kindness of the Alabama Polytechnic Institute. At times an adaptation of the stomatoscope with an ordinary microscope was employed.

TABLE I.—*Results of inoculating sugar-beet leaves of different ages* [a] *with Phoma betae and Phyllosticta betae*

Age of leaves and series No.	Phoma betae.				Phyllosticta betae.			
	Average number of stomata per sq. mm. of upper leaf surface.	Number of inoculations per leaf.	Condition of leaf four days after inoculation.	Number of infections per leaf.[b]	Average number of stomata per sq. mm. of upper leaf surface.	Number of inoculations per leaf.	Condition of leaf four days after inoculation.	Number of infections per leaf.[b]
Old leaves [c] (series 1).	62.8	3	Yellowing	2	71.0	3	Yellow	3
	73.8	3	Old mature	3	60.1	3	Old mature	3
	60.1	3	Yellowing	3	61.5	3	Old yellow	3
	68.3	3	Beginning to yellow	3	71.0	3	do	3
	79.2	3	Slightly yellowed	3	87.4	3	Mature	2
	76.5	3	Yellowing	2	82.0	3	Yellowing	3
	61.5	3	Old yellowing	3	90.2	3	Old mature	3
	73.8	3	Yellowing	3	71.0	3	Beginning to yellow	2
	60.1	3	do	3	90.2	3	Mature	3
	100.0	3	Old mature	2	76.5	3	Old yellow	0
	90.2	3	do	3	98.4	3	Mature	0
	65.6	3	Beginning to yellow	3	76.5	3	Yellowing	0
	82.0	3	Old mature	1	90.2	3	Old mature	9
	68.3	3	do	0	87.4	3	do	0
	85.2	3	Mature	0	71.0	3	Mature	0
	88.1	3	do	0	60.0	3		0
	82.0	3	do	0	82.0	3	Mature	0
	98.4	3	do	0	71.0	3	Old mature	0
	87.4	3	Old mature	0	84.7	3	Mature	0
	84.0	3	do	0	95.6	3	do	0
	82.0	3	do	0	82.0	3	Beginning to yellow	0
	82.0	3	do	0	82.0	3	Mature	0
	65.6	3	Mature	0	68.3	3	do	0
	84.7	3	Old mature	0	73.8	3	do	0
	82.0	3	do	0	92.9	3	do	0
	86.9	3	Mature	0	86.1	3	do	0
	82.0	3	Old mature	0	84.7	3	do	0
	73.8	3	do	0	84.7	3	do	0
	84.0	3	do	0	98.4	3	do	0
	95.6	3	do	0	92.9	3	do	0
					98.4	3	do	0
					87.4	3	Beginning to yellow	0
					79.2	3	Mature	0
	Total	90		34		99		25
Old leaves [c] (series 2).	65.6	4	Slightly yellow	1	84.7	4	Yellow	4
	60.1	4	Beginning to yellow and dying.	3	87.4	4	Still green	0
	82.0	4	Yellow	3	79.2	4	Slightly yellow	0
	76.5	4	do	4	82.0	4	Still green	0
	65.6	4	Yellow and dead	4	82.0	4	do	0
	79.2	4	Beginning to yellow	1	71.0	4	do	0
	84.7	4	Quite yellow	4	79.2	4	Rather more green than yellow.	2
	73.8	4	Yellowing	2	76.5	4	Yellowing	4
	57.4	4	do	4	84.7	4	Yellow and dying	4
	62.8	4	do	2	73.8	4	Still rather green	1
	87.4	4	Still somewhat green.	4	73.8	4	Yellow	4
	87.4	4	Still green	0	73.8	4	do	4
	Total	48		32		48		23
Young mature leaves [d] (series 3).	106.6	3		0	101.1	3		0
	103.8	3		0	106.6	3		0
	117.5	3		0	109.3	3		0
	103.8	3		0	110.7	3		0
	110.7	3		0				
	103.8	3		0				
	109.3	3		0				
	110.7	3		0				
	103.8	3		0				
	106.6	3		0				
	112.7	3		0				
	106.6	3		0				
	117.5	3		0				
	Total	39				12		

[a] Inoculations made on plants in the greenhouse of Bureau of Plant Industry, at Washington, D. C., on January 15 and 24, 1914.
[b] *Phoma betae* was reisolated from all infected spots that are indicated in series 1 and 2.
[c] The leaves in this series occupied the outermost position on the beet plants.
[d] The leaves designated as "young mature" were those occupying a medium position in the plant growth.

TABLE I.—*Results of inoculating sugar-beet leaves of different ages with Phoma betae and Phyllosticta betae*—Continued

Age of leaves and series No.	Phoma betae.				Phyllosticta betae.			
	Average number of stomata per sq. mm. of upper leaf surface.	Number of inoculations per leaf.	Condition of leaf four days after inoculation.	Number of infections per leaf.	Average number of stomata per sq. mm. of upper leaf surface.	Number of inoculations per leaf.	Condition of leaf four days after inoculation.	Number of infections per leaf.
Very immature or "heart" leaves [a] (series 4).	142.1	3	....	0	133.9	3	....	0
	153.0	3	....	0	174.9	3	....	0
	152.5	3	....	0	169.4	3	....	0
	139.4	3	....	0	143.5	3	....	0
	147.6	3	....	0	164.0	3	....	0
	153.0	3	....	0	169.4	3	....	0
	155.8	3	....	0	136.6	3	....	0
					147.6	3	....	0
					180.4	3	....	0
					144.8	3	....	0
					172.2	3	....	0
					232.3	3	....	0
					158.5	3	....	0
					172.2	3	....	0
	Total..	21	....	....	....	52	....	....

[a] "Heart" leaves occupied the central portion of the plant.

The data recorded in Table I (series 1, 3, and 4) show that the old, mature leaves of the sugar-beet plant or those leaves which are beginning to yellow are the only ones that are susceptible to *Phoma betae*. In order to corroborate this point, additional inoculations were made on leaves that were deemed to be in this condition at the time of inoculating. Out of 48 inoculations with *Phoma betae* 32 typical spots were produced, and with a like number of inoculations with *Phyllosticta betae* 23 infections developed. It will be noted in Table I (series 2) that the inoculations which did not produce infections were made on leaves that were evidently not correctly determined as to their degree of maturity, since, as a rule, they had not yet attained this age at the time the infections should have developed. The cultures in this series were obtained from reisolations of Phoma and Phyllosticta infections of series 1.

The original cultures used for the inoculations with *Phoma betae* were obtained from Mr. H. A. Edson, of the Bureau of Plant Industry, who isolated the organism from rotted sugar beets kept in storage at Longmont, Colo. Those of *Phyllosticta betae* used in the inoculations were obtained from heavily infected sugar-beet leaves that had been collected in Colorado during the growing season of 1913. Puncture inoculations used exclusively in this experiment were made from a suspension of the pycnospores in sterile water.

DISSEMINATION OF PHOMA BETAE

Such agencies as beet balls,[1] wind, irrigation water, insects, and dung play an important part in the distribution of *Phoma betae* in the field.

[1] Edson, H. A. Op. cit., p. 141.

From 44 plate cultures exposed (2 plates at a time) for 15 to 30 minutes near the open ground surface in various beet fields at different times from June 7 to September 10, 1912, inclusive, at Rocky Ford, Colo., 50 colonies of *P. betae* were obtained. The fungus was present in the air at temperatures which varied from 68° to 115° F. at the ground surface, with relative humidities from 39 to 71 per cent. These readings were taken during the time that the plates were exposed. The organism was not obtained from plates exposed during one night, an experiment which was of necessity limited. Its presence in the air seemed to be dependent on the humidity, a high humidity apparently causing the pycnidia to expel their spores, while a subsequent decrease in the relative humidity caused the spores to escape into the air.

At certain times *P. betae* occurs abundantly in irrigation water. This is particularly true late in August and early in September. The pycnidia are well formed on the leaves by this time, and if moistened they burst and many spores are expelled. Samples of irrigation water which was either standing between the rows or had drained to the lower portions of the beet fields about one day after irrigation yielded Phoma in several cases in the tests made in 1912. Thirty-three colonies of *P. betae* in plate cultures were obtained from 23 c. c. of water representing four such samples, while 3 c. c. of water flowing through a field yielded nine colonies in cultures.

Three species of insects have been found to be carriers of the fungus to only a slight extent. Two culture tests made with the moth of the beet webworm, *Loxostege sticticalis* L., yielded many colonies of *P. betae* in the latter part of July, while cultures made at later intervals gave negative results. Several tests made of the alkali beetle, *Monoxia juncticollis* Say, and the larvæ of the woolly bear (yellow), *Diacrisia virginica* Fab., yielded only a few colonies of the fungus.

Phoma betae may occur in abundance in the dung present in feed yards where beet tops have been fed. It is not to be concluded that the presence of the organism here indicates that it can survive a passage through the alimentary tract of cattle or sheep, but rather that the fungus is viable in dung after the ordinary method of feeding beet tops where they are not entirely consumed. In one test made early in January, 1913, 36 colonies of *P. betae* were obtained from nine small drops of strong manure decoction.

FACTORS INIMICAL TO VIABILILITY OF PHOMA BETAE IN BEET LEAVES

DRY HEAT

The thermal death point of *Phoma betae* in sugar-beet leaf tissue exposed for half an hour to dry heat is between 80° and 90° C. Seventy isolations of *Phoma betae* were made from spots on leaves exposed at 70° for half an hour. At 80° two colonies developed in cultures made from approximately the same amount of material, and at 90° and 100° none were obtained. It is evident, therefore, that the fungus would be rendered harmless when infected beet tops are dried in a pulp drier.

OVERWINTERING UNDER FIELD CONDITIONS

Phoma betae has been found to be present to a slight extent in the soil of old sugar-beet fields. It was isolated from samples of finely divided soil taken during March and April, 1912, from fields near Rocky Ford, Colo., which had been in sugar beets for several years. Four colonies were obtained from 0.05 gm. of a surface sample, while from cultures made from 0.25 gm. representing two different first-foot samples eight colonies were obtained. No growth of the fungus occurred in cultures made from second- and third-foot samples. Although the tests were continued throughout May, June, July, and the first part of August, no further isolations were made.

About the middle of October, 1912, sugar-beet leaves which were infected with *P. betae* were mixed with soil to the depth of 6 inches in a box and left exposed to outdoor weather changes. No cultures of the organism could be obtained from these leaves after 3 months. However, different results were obtained when the leaves were buried at various depths in the ground. It was found that the fungus was viable at the end of 3 months in leaves which had been buried at depths of 1 to 5 inches or had been kept in the interior of a pile of hayed beet leaves. The organism was isolated from leaves buried at depths of 1 to 5 inches after 5 months, but there was no development from the leaves buried 6 to 8 inches. At the end of 12 months no growth of *P. betae* was obtained at any depth, and the leaves were practically all disintegrated. However, the viability of the fungus was not impaired in dried leaves stored under herbarium conditions for over 2 years.

The maximum temperature of the air from October, 1912, to September, 1913, inclusive, the time of the overwintering experiment, varied from 4° to 102°, the minimum from −20° to 72° F. The maximum temperature of the ground at a depth of 5 inches from December, 1912, to May, 1913, inclusive, varied from 42° to 92°, the minimum from 22° to 51° F. During the 12 months of the experiment there was 9.34 inches of rainfall and snow, mostly during April, May, June, and July. There was no precipitation during November and December of 1912, but there occurred 0.2 inch in January, and 0.4 inch in October, 1913. During this time the lowest soil and air temperatures were registered.

It appears, then, that the results on the viability of the organism obtained from covering the leaves with soil in boxes are not comparable to those obtained under field conditions. The temperature of the air varies from that of the soil to such a degree that accurate results for field comparison can not be obtained in such an experiment. A period of one year seems sufficient to eliminate *Phoma betae* from infected beet-leaf material left in the field, although there is a probability that the fungus mycelium may remain dormant for a longer period of time in a sugar-beet root or "mother beet" stalk. The writers have found no evidence of a perfect stage of the organism.

The leaves for the outdoor-exposure experiments were buried in such a manner that examination was rendered convenient and accurate. The following method was suggested by Mr. W. A. Orton, Pathologist in Charge of Cotton- and Truck-Disease and Sugar-Plant Investigations, Bureau of Plant Industry. The soil was removed to the depth required and a piece of 2-inch mesh wire was placed on the exposed ground surface. The layer of leaves was then packed over this, another layer of wire added, and then soil to the depth desired. In examining the leaves at any time the layer of wire served to show the position of the leaves, and definite spots could be taken for cultural material. The effect of outdoor winter conditions on the viability of *Phoma betae* in infected beet tops is given in Table II.

TABLE II.—*Effect of different methods of overwintering on the viability of Phoma betae in infected beet leaves*

Method of treatment of infected leaves.	Length of exposure.	Number of cultures made.[a]	Number of isolations.[b]
	Months.		
Buried in soil in box	3	3	None.
Do	4	7	None.
Do	7	6	None.
Buried 1 inch in ground	6	21	Many.
Do	8	21	Few.
Do	10	4	Few.
Do	11½	8	None.
Buried 2 inches in ground	6	19	8.
Do	10	5	Few.
Do	11½	8	None.
Buried 3 inches in ground	6	21	Many.
Do	10	4	None.
Do	11½	8	None.
Buried 4 inches in ground	6	19	Many.
Do	10	6	None.
Do	11½	8	None.
Buried 5 inches in ground	6	20	Many.
Do	10	5	None.
Do	11½	8	None.
Buried 6 inches in ground	6	20	None.
Do	10	4	None.
Do	11½	8	None.
Buried 7 inches in ground	6	15	None.
Do	8	10	None.
Do	10	4	None.
Do	11½	8	None.
Buried 8 inches in ground	6	20	None.
Do	8	10	None.
Do	10	4	None.
Do	11½	8	None.
Interior of "hayed" pile of beet tops	3½	30	6.
Do	4	8	1.
Do	6	6	2.
Do	11½	8	None.
Left in field on surface of ground	5½	11	6.

[a] String-bean agar was used for all cultures.
[b] The number of spots used for each cultural plate varied from 1 to 4 or 5.

ENSILAGE

The process of siloing infected beet tops has been found to be sufficient to kill *Phoma betae*. In the ensilage experiments carried on during the winters of 1912 and 1913 it was ascertained that the organism was viable at the time the silage was made, but could not be isolated after the tops had been ensiled for two months. A medium composed of somewhat diluted silage material was also inimical to the growth of the fungus. Detailed data will be published later in connection with the relation of *Cercospora beticola* to ensiled beet tops.

SUMMARY

A typical spot of *Phoma betae* (Oud.) Fr. is light brown in color, 1 to 2 cm. in diameter, and has scattered over its surface numerous pycnidia, at times concentrically arranged. Such spots on a normal beet plant usually appear during July and August on the old leaves near the ground. If the plant is physiologically low, all except the heart leaves may become infected.

Phoma betae produces a characteristic infection on leaves that have a stomatal count of 60 to 100 per sq. mm. of upper leaf surface.

The pycnospores of the fungus may be disseminated by such agencies as beet balls, wind, irrigation water, insects, and dung.

The thermal death point of *Phoma betae* in the leaf tissue exposed for one-half hour to dry heat is between 80° and 90° C. The fungus is dead in infected leaves after three months' storage in soil in boxes exposed to outdoor conditions, while its life becomes extinct in leaves buried in the ground only after five to eight months, depending on the depth of cover. The fungus can not survive the process of ensiling the beet tops.

PLATE XXVII

An old leaf of a sugar-beet plant showing typical spots of *Phoma betae*.